RESEARCH ARTICLE

Arbuscular mycorrhizal symbiosis can counterbalance the negative influence of the exotic tree species *Eucalyptus camaldulensis* on the structure and functioning of soil microbial communities in a sahelian soil

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Abstract

The hypothesis of the present study was that bacterial communities would differentiate under *Eucalyptus camaldulensis* and that an enhancement of arbuscular mycorrhizal (AM) density would minimize this exotic plant species effect. Treatments consisted of control plants, preplanting fertilizer application and AM inoculation. After 4 months of culture in autoclaved soil, *E. camaldulensis* seedlings were either harvested for growth measurement or transferred into containers filled with the same soil but not sterilized. Other containers were kept without *E. camaldulensis* seedlings. After 12 months, effects of fertilizer amendment and AM inoculation were measured on the growth of *Eucalyptus* seedlings and on soil microbial communities. The results clearly show that this plant species significantly modified the soil bacterial community. Both community structure (assessed by denaturing gradient gel electrophoresis profiles) and function (assessed by substrate-induced respiration responses including soil catabolic evenness) were significantly affected. Such changes in the bacterial structure and function were accompanied by disturbances in the composition of the herbaceous plant species layer. These results highlight the role of AM symbiosis in the processes involved in soil bio-functioning and plant coexistence and in afforestation programmes with exotic tree species that target preservation of native plant diversity.

Introduction

Over the last century, plantation forestry using exotic trees has developed as an integral and crucial part of many national economies and environmental programmes. Tree species such as *Pinus* spp., *Eucalyptus* spp. and *Acacia* spp. have been introduced outside their natural range throughout the 18th and 19th centuries, and have been intensively planted in the framework of afforestation programmes or for agroforestry purposes since the second half of the 20th century (Evans, 1982). Fast-growing exotic trees have been used to provide wood or fodder for browsing livestock, prevent desertification, and curtail wind and rain erosion, because of their ability to grow rapidly under harsh conditions (Parrotta, 1993). However, this widespread anthropogenic dispersal of exotic organisms has raised growing

concern over their potentially devastating ecological impacts on native organisms. Several well-documented studies have shown the hazards that can result from an introduced tree or woody shrub that could become invasive by altering ecological interactions among native species in the invaded zone (Mooney & Hobbs, 2000; Rejmanek, 2000; Callaway & Ridenour, 2004). A general definition has been suggested by Shine *et al.* (2000): an invasive species is considered as an alien species that becomes established in natural or semi-natural ecosystems or habitat and is 'an agent of change and threatens native biological diversity'. Exotic plants could threaten ecosystems, habitats or species for different reasons, such as a higher performance in a new site (Thébaud & Simberloff, 2001), direct chemical interference (allelopathic effect) with native plant ecosystem influencing succession, dominance, community structure and composition, vegetation dynamics (del Moral & Muller, 1970), or resistance capacity of native vegetation to invasion (Hobbs & Huenneke, 1992; Levine & D'Antonio, 1999). It has also been suggested that exotic plants could interact with soil microbial communities and disrupt mutualistic associations between existing ecological associations within native communities (Richardson *et al.*, 2000; Callaway & Ridenour, 2004). Among soil microbial communities, arbuscular mycorrhizal (AM) fungi form a key component of the sustainable soil–plant system (Schreiner *et al.*, 2003; Johansson *et al.*, 2004). AM symbiosis has been usually considered as an association between host plant and endophyte only. More recently this symbiotic process has been recognized to influence soil development as much as plant development (Schreiner & Bethlenfalvay, 1995; Schreiner *et al.*, 2003; Duponnois *et al.*, 2005). It has been reported that AM fungi affect the diversity of plant communities (van der Heijden *et al.*, 1998; Klironomos *et al.*, 2000; O'Connor *et al.*, 2002) and influence relationships between plants (West, 1996; Marler *et al.*, 1999; van der Heijden *et al.*, 2003).

In its native distribution area, *Eucalyptus camaldulensis* is found over most of the Australian mainland, except southern Western Australia, south-western South Australia and the eastern coastal areas of Queensland, New South Wales and Victoria (Chippendale, 1988). In these regions, this tree species is generally dominant in the community, commonly forming pure open forests or woodlands (Costermans, 1989). It is considered to be one of the most widely planted eucalypts in the world and plantations occur in Argentina, California, Egypt, Morocco, Senegal, etc (NAS, 1980). However, *Eucalyptus* are extremely damaging ecologically to many native plant species. The annual vegetation adjacent to naturalized stands of *E. camaldulensis* is inhibited severely and annual herbs rarely survive to maturity when *Eucalyptus* litter accumulates. In addition, some concerns about the effects of *Eucalyptus* plantations are related in terms of depletion of nutrients, acidification and excessive water utilization of the species (Couto & Betters, 1995). However, the influence of this exotic tree species on the structure and function of microbial communities is unknown outside its home range. In particular, the possibility that its establishment could cause changes on AM fungal functioning has not been studied in its introduction area.

The aims of this study were to test under glasshouse conditions the impact of *E. camaldulensis* on bacterial functional capabilities and more particularly on AM fungi density. We hypothesized that bacterial communities would differentiate under this exotic tree species, and that this influence would modify bacterial functional diversity. We further hypothesized that an enhancement of AM density (through controlled AM fungal inoculation of *E. camaldulensis* seedlings) would minimize the effect of this exotic plant species on the annual vegetation through a well-developed mycelium network that contributes to the development of the adjacent plant species. Finally, we tested the hypothesis that AM symbiosis could regulate plant species coexistence and change competitive relationships between plants.

Materials and methods

Plant and fungal inoculum

Seeds of *E. camaldulensis* Dehn. (Provenance Kambouinsé, Burkina Faso) were surface-sterilized with $HgCl_2$ for 10 min, thoroughly rinsed and imbibed for 24 h in sterile distilled water (120 °C, 20 min). They were then transferred aseptically in Petri dishes filled with 1% (w/v) water agar. After 8 days of incubation in the dark at 25 °C, the germinating seeds were used when rootlets were 1–2 cm long.

The AM fungus *Glomus intraradices* Schenk & Smith (DAOM 181602, Ottawa Agricultural Herbarium) was propagated on leek (*Allium porrum* L.) on a calcined clay (particle size average 5 mm), Oil-Dry US-special Ty/IIIR (Oil-Dri company, Chicago) under greenhouse conditions. After 12 weeks of culturing, the leek plants were uprooted and gently washed. Roots were then cut into 0.5-cm pieces bearing around 250 vesicles cm^{-1}. Nonmycorrhizal leek roots prepared as above were used for the control treatment without AM inoculation.

Experimental design

The germinated seeds of *E. camaldulensis* were individually grown in 1-L capacity pots filled with an autoclaved sandy soil (120 °C, 60 min) collected in an experimental station localized at Gampela (20 km from Ouagadougou, Burkina Faso). After autoclaving, the physico-chemical characteristics of the soil were as follows: pH (H_2O) 5.6, clay 4.6%, fine silt 0.0%, coarse silt 0.8%, fine sand 25.5%, coarse sand 69.1%, carbon 2.04%, total nitrogen 0.04%, Olsen phosphorus 4.3 mg kg^{-1}, total phosphorus 116 mg kg^{-1}. Three

treatments were carried out: control, preplanting fertilizer application and AM inoculation with *G. intraradices*. In AM inoculation, 1 g of fresh mycorrhizal leek roots was placed in a hole (1×5 cm) in the soil of each pot. Treatments without fungus (control and preplanting fertilizer application) received nonmycorrhizal leek roots at the same rate. Preplanting fertilizer application was applied by adding 0.5 g Osmocote^{TM} granulates into the soil of each pot (N/P/K, 11 : 8 : 17). This fertilization rate has been calculated according to the results of previous experiments (data not published). All of the planted pots were kept in a greenhouse (daylight *c.* 12 h, average daily temperature 25 °C) and were watered with tap water (pH = 6.5) as frequently as necessary to keep the soil moist. They were arranged in a randomized complete block design with 10 replicates per treatment.

After culture for 4 months, five plants were chosen randomly from each treatment. The *E. camaldulensis* plants were uprooted, the height and the oven dry weight (1 week at 65 °C) of the shoot were measured. For each plant, the entire root system was gently washed, cleared and stained according to the method of Phillips & Hayman (1970). The root pieces were placed on a slide for microscopic observation at $\times 250$ magnification (Brundrett *et al.*, 1985). About 100 1-cm root pieces were observed per plant. The extent of AM colonization was expressed as a percentage of mycorrhizal root pieces. Then, the stained roots were collected and weighed for each plant (1 week, 65 °C).

For each treatment, the remaining five plants were transferred with their cultural substrate into 175-L capacity containers ($50 \times 50 \times 70$ cm) filled with the same soil as before but not disinfected. This soil was thoroughly mixed to ensure the homogeneity of the seed bank in the soil. Five other containers, prepared as described previously, were kept without *E. camaldulensis* seedlings. The containers were arranged in a complete randomized block design and placed outside in the IRD (Institut de Recherche pour le Développement) experimental station of Ouagadougou (Burkina Faso) in a clean area. The plants were grown without any solar protection and at ambient temperature from 20 °C to 40 °C with daily watering.

After 12 months' culture, the height of the *E. camaldulensis* trees was measured. Herbaceous plant species naturally growing in each pot were identified and their shoot and biomasses were determined (1 week, 65 °C). The composition of herbaceous layer was assessed using species richness and Shannon's diversity index (Krebs, 1989) based on individual species total biomass data. On each *E. camaldulensis* plant, the leaves and the stem were divided and their oven-dried weights were determined (2 weeks, 65 °C). Then *E. camaldulensis* plants were uprooted and their root systems were gently washed. Two grams of fresh root was randomly collected along the root system of each plant to evaluate the intensity of mycorrhizal symbiosis. The extent of AM colonization was expressed as described before. Then the entire root systems were oven-dried (1 week, 65 °C) and weighed. The soil collected from each container was carefully mixed and 10-kg subsamples were taken and kept at 4 °C for further measurements.

Soil analysis

All soils sampled from each pot were characterized by measuring pH, total soil organic C after dichromate oxidation, total organic N by the Kjeldahl method, total soil P by acid extraction and soluble P by the Olsen–Dabin method.

Microbial community structure

The genetic structure of the soil total bacterial communities in each treatment was assessed by PCR-DGGE (denaturing gradient gel electrophoresis). PCR amplification targeting total soil 16S rRNA gene bacterial community was realized with the eubacterial primer pair 338f-GC (Ovreas *et al.*, 1997) and 518r (Muyzer *et al.*, 1993). Total genomic DNA extraction and PCR amplification were performed as described previously (Assigbetse *et al.*, 2005). DGGE fingerprints were scored by the presence or absence of comigrating bands, independent of intensity. Profile similarity was calculated by determining Dice's coefficient for the total number of bands. Dendrograms were constructed by using the unweighted pair group method with arithmetic averages (Assigbetse *et al.*, 2005).

Microbial community function

The function of soil microbial communities was assessed by determining their patterns of *in situ* catabolic potential to provide microbial functional diversity in soil treatments (Degens & Harris, 1997). Different amino acids, carbohydrates, organic acids and amides were added to the soil and the short-term respiration responses were determined (Degens & Harris, 1997; Degens *et al.*, 2001). One gram of equivalent dry weight soil collected from each container was suspended in 2 mL substrate solution (West & Sparling, 1986) in 10-mL bottles. CO_2 production from basal production respiratory in the soil samples was measured by adding 2 mL sterile distilled water to 1 g of the equivalent dry weight of soil. After the addition of the substrate solutions to the soil samples, the bottles were immediately sealed with a Vacutainer stopper and incubated at 28 °C for 4 h in darkness. CO_2 fluxes from the soils were measured using an infrared gas analyser (IRGA) (Polytron IR CO_2, Dräger^{TM}) in combination with a thermal flow meter (Heinemeyer *et al.*, 1989). The results were expressed as $\mu g\, CO_2\, g^{-1}\, soil\, h^{-1}$. They were 10 amino acids (L-phenylalanine, L-glutamine, L-serine, L-arginine, L-asparagine,

L-histidine, L-lysine, L-glutamic acid, L-tyrosine, L-cystein), three carbohydrates (D-glucose, D-mannose, sucrose), three amides (D-glucosamine, *N*-methyl-D-glucamine, succinamide) and 16 carboxylic acids (ascorbic acid, citric acid, fumaric acid, gluconic acid, quinic acid, malonic acid, formic acid, α-ketoglutaric acid, α-ketobutyric acid, succinic acid, tartaric acid, uric acid, oxalic acid, gallic acid, malic acid, hydroxybutyric acid). The amines and amino acids were added at 10 mM, whereas the carbohydrates were added at 75 mM and the carboxylic acids at 100 mM (Degens & Vojvodic-Vukovic, 1999). The catabolic evenness (*E*) was calculated to determine the catabolic diversity of soil treatments. It describes the variability of used substrates amongst the range of the substrates tested and is calculated using the Simpson–Yule index $E = p/p_i^2$ with p_i = respiration as the response to individual substrates/total respiration activity induced by all substrates (Magurran, 1988).

Assessment of the mycorrhizal soil infectivity

AM hyphal length was measured on membrane filters according to Jakobsen & Rosendahl (1990). Soil samples (2 kg) were collected from four containers randomly chosen in each treatment. Four dilutions were made of each soil sample by thoroughly mixing the original soil in 1 : 4 proportions with the same soil but autoclaved (120 °C, 40 min). Ten replicates were prepared for each dilution. Seeds of *Sorghum vulgare* Pers. were surface sterilized with 10% sodium hypochlorite, washed with sterile distilled water (120 °C, 20 min) and pregerminated for 2 days in Petri dishes on humid filter paper. One germinated seed was then transplanted into each of 100 mL pots filled with 100 g of different soil solution. The pots were placed in a glasshouse under natural light (daylight *c.* 12 h, mean temperature 30 °C) and watered daily with deionized water. After 40 days of growth, seedlings were uprooted and their entire root systems were washed under tap water. On each plant, the extent of AM colonization was assessed as described above.

Statistical analysis

Data were treated with one-way ANOVA. Means were compared using the Newman–Keuls test ($P < 0.05$). The percentages of the mycorrhizal colonization were transformed by arcsin(sqrt) before the statistical analysis.

Between-group analysis (BGA, Dolédec & Chessel, 1989; Culhane *et al.*, 2002) was used to analyse the substrate-induced respiration (SIR) profiles of soil samples submitted to the four treatments: soil without *E. camaldulensis*, control, preplanting fertilizer application and *G. intraradices* inoculation. BGA is an ordination method that can be used as a robust alternative to the well-known discriminant analysis (Huberty, 1994). As with the discriminant analysis, the aim is to classify cases into *a priori* groups. However, the discriminant analysis has several drawbacks: the number of cases must be high compared to the number of variables (several times higher). Computations cannot be performed when the number of cases is lower than the number of variables. Moreover, the discriminant analysis must meet the same assumptions as the ANOVA (normal distribution, homogeneity of variances). BGA makes no assumption on data distribution and variances, and the ratio of case number to variable number is not constrained. Specifically, BGA can be used even when the number of cases is lower than the number of variables, which is the case here (20 soil samples and 32 SIR substrates). A permutation test (Monte-Carlo method) allows us to check the statistical significance of the between-groups differences. The free ADE4 software (Thioulouse *et al.*, 1997) was used to perform BGA computations

For the assessment of the mycorrhizal soil infectivity, the relationships between the extent of AM colonization and soil dilutions were analysed using mixed effects linear regression models. The *p*-values for model fits were obtained by ANOVA (Venables & Ripley, 2002). Computations were conducted with the R software (R Development Core Team, 2006), using the 'NLME' package (Pinheiro & Bates, 2000). In the regression models, the fixed effects were the soil type: without *E. camaldulensis*, control, preplanting fertilizer application and *G. intraradices* inoculation and the level of dilution (nondisinfected soil/disinfected soil: 1, 1/4, 1/16, 1/64). The random effect was the repetitions for each percentage of nonsterilized soil.

Results

Eucalyptus and herbaceous species development

After 4 months' culture in the disinfected soil, AM inoculation significantly increased the growth of *E. camaldulensis* seedlings by factors of 1.40, 1.79 and 1.83 for height, shoot and root dry weight, respectively, compared with the control (Table 1). No significant differences were recorded between the preplanting fertilizer application and *G. intraradices* treatments (Table 1). No AM structures (vesicles, hyphae or arbuscules) were observed in the preplanting fertilizer application and the control treatments, whereas the extent of AM colonization was 45.6% for *G. intraradices*-inoculated seedlings.

After 12 months' further culture in nondisinfected soil, the stimulating effects of the fertilizer amendment and *G. intraradices* inoculation were recorded for all the measured parameters (height, leaf and stem biomass, root biomass; Table 2). The growth of *E. camaldulensis* seedlings was not significantly different in the preplanting fertilizer

Table 1. Growth response of *Eucalyptus camaldulensis* seedlings and AM colonization in soils inoculated with *Glomus intraradices* or fertilized after 4 months culture in a disinfected soil (means of 10 replicates)

Treatments	Height (cm)	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	AM colonization (%)
Control	32.3 (1.2)* a†	852 (23.6) a	326 (15.3) a	0
FA‡	45.3 (1.9) b	1532 (42.6) b	598 (12.6) b	0
G. intraradices	43.6 (2.3) b	1499 (39.7) b	624 (15.4) b	45.6 (5.9)

*Standard error of the mean.
†Data in the same column followed by the same letter are not significantly different according to the Newman–Keuls test ($P < 0.05$).
‡Preplanting fertilizer application.

Table 2. Growth response, AM colonization of *Eucalyptus camaldulensis* grown in soils inoculated with *Glomus intraradices* or fertilized, total above and below-ground biomass, plant species richness, Shannon index of the herbaceous cover recorded in each treatment after 12 months' culture in a nondisinfected soil

	Treatments			
	WEC*	Control	FA†	*G. intraradices*
Height (cm)		144 (1.8) ‡ a §	196 (13.7) b	166 (5.3) b
Leaf biomass (g dry weight)		52.5 (1.3) a	62.9 (2.9) b	72.1 (7.4) b
Stem biomass (g dry weight)		68.3 (3.2) a	116 (7.4) b	96.1 (5.1) b
Total shoot biomass (g dry weight)		121 (4.3) a	179 (9.2) b	168 (9.1) b
Root biomass (g dry weight)		50.5 (6.1) a	82.9 (6.6) b	73.1 (6.4) b
AM colonization (%)		38.1 (2.8) a	37.7 (3.3) a	52.1 (3.7) b
Hyphal length (m g^{-1} soil)	3.9 (0.6) b	2.3 (0.2) a	2.8 (0.5) a	4.2 (0.3) b
Above-ground biomass (g dry weight)	21.3 (3.5) c	1.94 (0.45)‡ a §	1.04 (0.35) a	9.51 (3.36) b
Below-ground biomass (g dry weight)	4.2 (1.1) d	0.25 (0.06) b	0.09 (0.03) a	0.85 (1.13) c
Plant species richness	4.8 (0.49) c	2.8 (0.37) b	1.2 (0.2) a	3.0 (0.63) b
Shannon index	0.97 (0.1) c	0.63 (0.15) b	0.11 (0.11) a	0.57 (0.13) b

*Without *E. camaldulensis* seedlings.
†Preplanting fertilizer application.
‡SEM.
§Data in the line followed by the same letter are not significantly different according to Newman–Keuls test ($P < 0.05$).

application and *G. intraradices* treatments (Table 2). AM colonization was significantly greater for the AM-inoculated seedlings than in the other treatments (Table 2). The length of external hyphae was significantly greater in the soil kept without *E. camaldulensis* and in the *G. intraradices* treatment than in the other treatments (control and pre-planting fertilizer application; Table 2).

Above- and below-ground herbaceous biomasses ranged among the treatments as follows: without *E. camaldulensis* > *G. intraradices* > control > preplanting fertilizer application treatment (Table 2). The preplanting fertilizer application decreased above- and below-ground biomasses by $\times 0.05$ and $\times 0.02$, respectively, compared to the treatment without *E. camaldulensis* (Table 2). The highest plant species richness and Shannon index were recorded in the treatment without *E. camaldulensis*, whereas the lowest were found in the preplanting fertilizer application treatment (Table 2). No significant differences were found between the control and preplanting fertilizer application treatments.

Fourteen herbaceous species were found within all the treatments (Table 3). For the above-ground biomass, seven species were predominant in the absence of *E. camaldulensis* (*Dactyloctenium aegyptium*, *Digitaria horizontalis*, *Euphorbia hirta*, *Eucalyptus viridis*, *Leucas martinicensis*, *Spermacoce radiata Sesamoides* sp. and *Spermacoce chaetocephala*), whereas the shoot growth of two other species (*Corchorus tridens* and *Polycarpaea corymbosa*) was larger in the *G. intraradices* treatment (Table 3). No significant differences were recorded for plant species in the other treatments except for *Eragrostris tremula* with a higher above-ground biomass in the absence of *E. camaldulensis* and in the *G. intraradices* treatment than in the others (Table 3). Highest below-ground biomasses were recorded in the treatment without *E. camaldulensis* with the following plant species: *Dactyloctenium aegyptium*, *Dactyloctenium horizontalis*, *E. tremula*, *Eucalyptus hista*, *E. viridis*, *L. martinicensis*, *Sesamoides* sp., *S. radiata* and *S. chaetocephala* whereas the highest below-ground biomasses were found in the *G. intraradices* treatment for *C. tridens*, *Oxalis corymbosa* and *P. corymbosa* (Table 4). No significant differences were detected within all the treatments with *C. siamea* and *S. festivus* (Table 4).

Table 3. Above-ground biomass (mg dry weight) of individual plant species recorded in each treatment after 12 months' culture in a nondisinfected soil

		Treatments			
Plant species	Families	WEC*	Control	FA†	*Glomus intraradices*
Cassia siamea Lam.	*Fabaceae*	0 a	20.4 (13.6)‡ a§	0 a	0 a
Corchorus tridens L.	*Tiliaceae*	179 (48.2) c	118 (8.5) b	0 a	942 (42.6) d
Dactyloctenium aegyptium (L.) Willd	*Poaceae*	1043 (74.7) c	23.5 (3.5) b	0 a	11.2 (8.9) b
Digitaria horizontalis Willd	*Poaceae*	5211b (95.9) d	112.8 (2.8) b	0 a	1021 (25.4) c
Eragrostis tremula Hochst. ex Steud	*Poaceae*	6690 (696) b	687 (36.9) a	741 (42.3) a	6272 (26.1) b
Euphorbia hirta L.	*Euphorbiaceae*	881 (50.7) c	256 (29.1) b	0 a	113 (97.3) b
Euphorbia viridis Klotzsch & Garcke ex Klotzsch	*Euphorbiaceae*	384 (30.3) b	0 a	0 a	0 a
Leucas martinicensis (Jacq.) R. Br.	*Lamiaceae*	627 (27.6) b	0 a	0 a	10.7 (5.7) a
Oldenlandia corymbosa L.	*Rubiaceae*	0 a	0 a	59.1 (59.1) a	562 (562) a
Polycarpaea corymbosa (L.) Lam	*Caryophyllaceae*	119.2 (6.5) b	0 a	0 a	291 (91.6) c
Sesamoides sp.	*Resedaceae*	735 (35.1) b	0 a	0 a	0 a
Spermacoce radiata Sieber ex DC.	*Rubiaceae*	3201 (205) c	666 (166) b	113 (12.9) a	106 (57.6) a
Spermacoce chaetocephala DC.	*Rubiaceae*	2248 (475) b	0 a	0 a	0 a
Sporobolus festivus A. Rich.	*Poaceae*	0 a	0 a	122 (122) a	0 a

*Without *Eucalyptus camaldulensis* seedlings.
†Preplanting fertilizer application.
‡SEM.
§Data in the line followed by the same letter are not significantly different according to Newman–Keuls test ($P < 0.05$).

Table 4. Below-ground biomass (mg dry weight) of individual plant species recorded in each treatment after 12 months' culture in a nondisinfected soil

		Treatments			
Plant species	Families	WEC*	Control	FA†	*Glomus intraradices*
Cassia siamea Lam.	*Fabaceae*	0 a	19.1 (16.8)‡ a§	0 a	0 a
Corchorus tridens L.	*Tiliaceae*	60.9 (6.9) b	6.48 (5.3) a	0 a	119 (12.9) c
Dactyloctenium aegyptium (L.) Willd	*Poaceae*	167 (55.7) b	1.30 (0.6) a	0 a	1.48 (0.78) a
Digitaria horizontalis Willd	*Poaceae*	103 (7.8) d	9.34 (3.4) b	0 a	44.9 (7.5) c
Eragrostis tremula Hochst. ex Steud	*Poaceae*	1678 (16.7) d	73.2 (2.2) b	54.3 (3.7) a	519 (97.4) c
Euphorbia hirta L.	*Euphorbiaceae*	386 (3.5) d	87.5 (6.9) c	0 a	37.6 (4.9) b
Euphorbia viridis Klotzsch & Garcke ex Klotzsch	*Euphorbiaceae*	16.9 (7.9) b	0 a	0a	0 a
Leucas martinicensis (Jacq.) R. Br.	*Lamiaceae*	177 (17.2) b	0 a	0 a	1.9 (1.9) a
Oldenlandia corymbosa L.	*Rubiaceae*	0 a	0 a	8.4 (8.4) a	595 (54.5) b
Polycarpaea corymbosa (L.) Lam	*Caryophyllaceae*	8.60 (5.5) a	0 a	0 a	58.6 (5.6) b
Sesamoides sp.	*Resedaceae*	423 (49.2) b	0 a	0 a	0 a
Spermacoce radiata Sieber ex DC.	*Rubiaceae*	555 (59.7) c	56.7 (5.7) b	2.26 (2.26) a	16.6 (16.6) a
Spermacoce chaetocephala DC.	*Rubiaceae*	847 (87.1) b	0 a	0 a	0 a
Sporobolus festivus A. Rich.	*Poaceae*	0 a	0 a	9.7 (2.7) a	0 a

*Without *Eucalyptus camaldulensis* seedlings.
†Preplanting fertilizer application.
‡SEM.
§Data in the line followed by the same letter are not significantly different according to Newman–Keuls test ($P < 0.05$).

Soil and bacterial communities analysis

After 12 months' further culture in nondisinfected soil, the chemical characteristics of soils (pH, total soil organic C, total organic N, total soil P and soluble P) were not significantly different within all the treatments (data not shown).

The 16S rRNA gene-DGGE patterns of the total bacterial communities from the control, preplanting fertilizer application, *G. intraradices* and without *E. camaldulensis* treatments are presented in Fig. 1. Numerous DGGE bands of various intensities that resulted from differences between the 16S rRNA gene sequences of different bacterial species were detected. They ranged in mobility *c*. from 30% to 60% of the denaturing gradient with different DGGE patterns between each treatment (Fig. 1). The PCR-DGGE displayed complex profiles reflecting changes in the structure of the total bacterial communities under the different treatments.

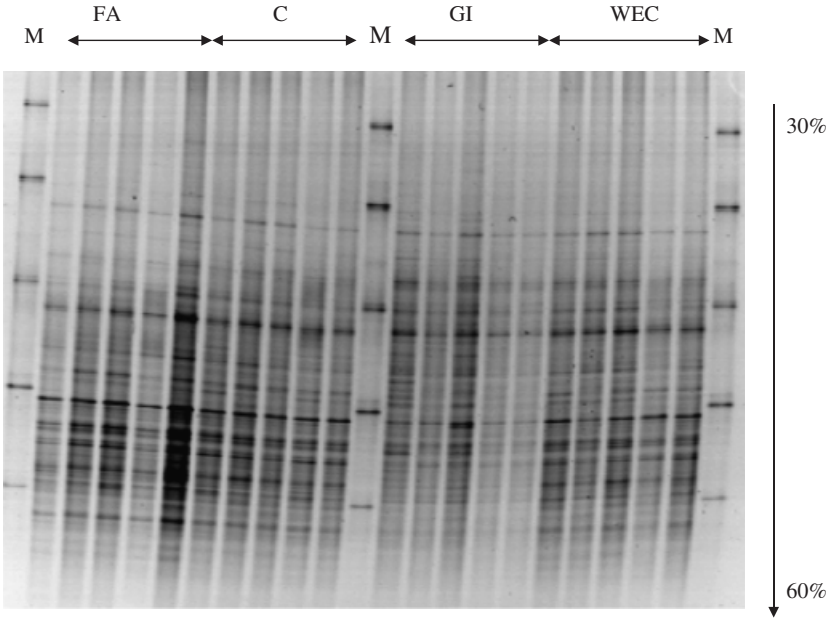

Fig. 1. DGGE of 16S rRNA gene of total soil bacterial communities in the soil without *Eucalyptus camaldulensis* (WEC), control (C), preplanting fertilizer application (FA) and AM inoculation with *Glomus intraradices* (GI). Migration was performed with an 8% acrylamide gel. Percent values indicate the percentage of denaturants at each position. M, marker, composed of PCR products generated from the following bacteria strains (from top to bottom): *Clostridium* sp., *Enterococcus cecorum*, *Bacteroidaceae* sp., *Pseudomonocardia zijingensis, Actinobacteria* sp.

Nevertheless, the banding patterns from different soil treatments shared most of the DGGE bands, indicating that a common bacterial population colonized the soil regardless of those treatments. The soils collected from the preplanting fertilizer application and control treatments displayed relatively common complex banding patterns while the DGGE banding profiles revealed by soils kept without *E. camaldulensis* and from *G. intraradices* treatment seemed to be related (Fig. 1). Hierarchical cluster analysis of the DGGE results for the five repetitions (1, 2, 3, 4, 5) and the four treatments (control, preplanting fertilizer application, *G. intraradices* and without *E. camaldulensis* treatments) are presented in Fig. 2. The scale gives the distances, computed by the Ward method, between repetitions and tree nodes. The analysis displayed four main clusters grouped into two related branches. The results indicated that the community structure of the *G. intraradices* and without *E. camaldulensis* treatments appeared to be related while control and preplanting fertilizer application treatments were grouped in the same branch (Fig. 2).

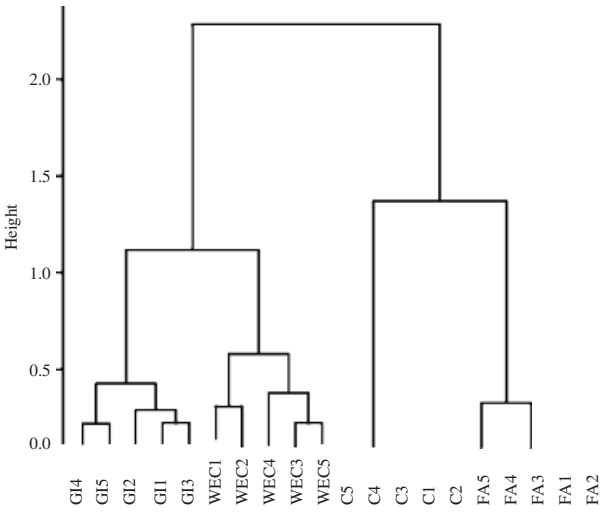

Fig. 2. Similarities between PCR-DGGE profiles obtained from bacterial communities in each soil treatments: without *Eucalyptus camaldulensis* seedlings (WEC), control (C), preplanting fertilizer application (FA) and AM inoculation with *Glomus intraradices* (GI).

The permutation test of BGA showed that the four treatments gave very different substrate-induced respiration profiles ($P < 0.001$). The 20 soil samples were grouped by stars according to the soil treatments (Fig. 3b). The four treatments were very well separated, with the soil kept without *E. camaldulensis* on the left, preplanting fertilizer application in the upper right, *G. intraradices* in the middle, and the control in the lower right of the figure. The substrates preferentially used in samples collected from the control treatment were tartaric acid and cystein (Fig. 3a). Conversely, the substrate preferentially used in preplanting fertilizer application soil samples was ketoglutaric acid,

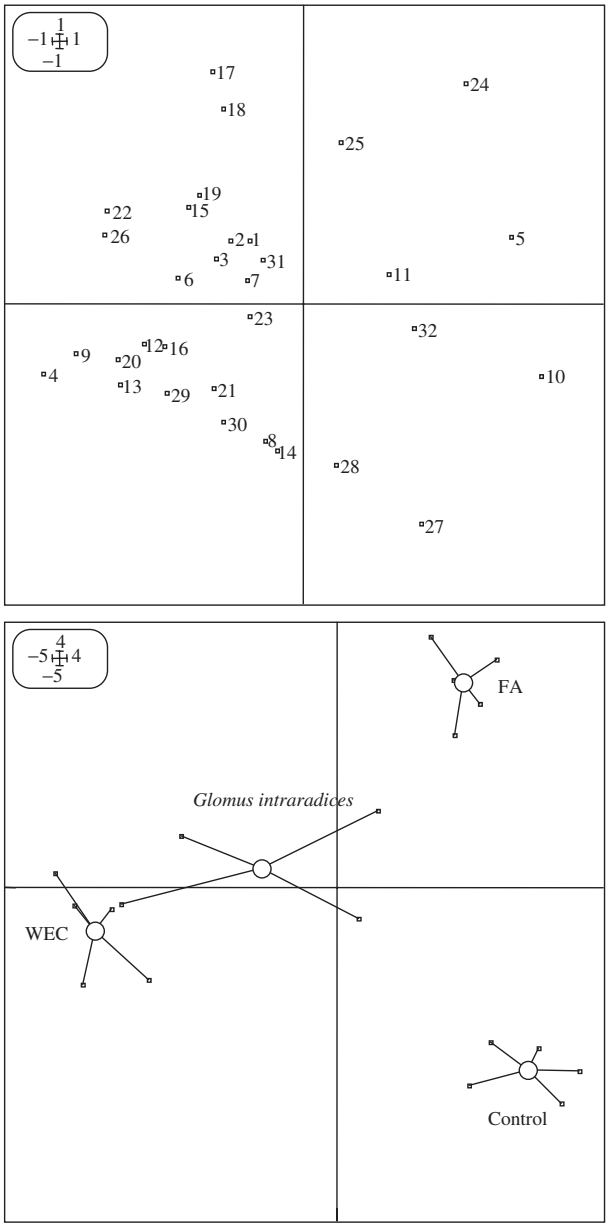

Fig. 3. Between-group analysis (BGA) of the SIR responses with respect to the soil treatments. WEC, without *Eucalyptus camaldulensis* seedlings. FA, preplanting fertilizer application. 1, L-phenylalanine; 2, L-glutamine; 3, L-serine; 4, L-arginine; 5, L-asparagine; 6, L-histidine; 7, L-lysine; 8, L-glutamic acid; 9, L-tyrosine; 10, L-cystein; 11, D-glucose; 12, D-mannose; 13, sucrose; 14, D-glucosamine; 15, *N*-methyl-D-glucamine; 16, succinamide; 17, ascorbic acid; 18, citric acid; 19, fumaric acid; 20, gluconic acid; 21, quinic acid; 22, malonic acid; 23, formic acid; 24, α-ketoglutaric acid; 25, α-ketobutyric acid; 26, succinic acid; 27, tartaric acid; 28, uric acid; 29, oxalic acid; 30, gallic acid; 31, malic acid; 32, DL-α-hydroxy-butyric acid.

whereas they were arginine and tyrosine for the soil samples kept without *E. camaldulensis* (Fig. 3a).

The catabolic evenness in the *G. intraradices* treatment was significantly higher than those recorded in the other treatments and the lowest catabolic evenness was found in the preplanting fertilizer application treatment (Table 5). The average SIR responses to amides and carbohydrates were significantly higher in soil from the treatment without *E. camaldulensis* than in the other treatments (Table 5). A significantly higher response to carboxylic acids was recorded in soil from the preplanting fertilizer application treatment (Table 5). The average SIR response to amino acids ranged among the treatments as follows: without *E. camaldulensis* > preplanting fertilizer application > control and *G. intraradices* treatments (Table 5).

The regression model between the extent of AM colonization and soil dilutions for the four different treatments showed that the treatment and the percentage of soil dilution both had very strong effects ($P < 0.0001$), but their interaction was nonsignificant ($P = 0.2322$) (Fig. 4). The differences between the control and the preplanting fertilizer application treatments ($P = 0.3007$) and between the treatments with *G. intraradices* and without *E. camaldulensis* ($P = 0.9748$) were not significant. However, the differences between the control and the *G. intraradices* treatments ($P = 0.0031$) and between the preplanting fertilizer application treatment and that without *E. camaldulensis* ($P < 0.0001$) were extremely significant.

Relationships between microbial functions, mycorrhizal soil infectivity, *Eucalyptus* and herbaceous species development

Significant positive correlations were recorded between hyphal length and above-ground biomass ($r = 0.64$, $P = 0.002$) and below-ground biomass of the herbaceous layer ($r = 0.47$, $P = 0.03$), hyphal length and catabolic evenness of the soil treatments ($r = 0.45$, $P = 0.04$). The root biomass of noninoculated *E. camaldulensis* plants (control and preplanting fertilizer application treatments) was negatively correlated to the above-ground biomass ($r = 0.82$, $P = 0.0002$) and below-ground biomass of the herbaceous layer ($r = 0.74$, $P = 0.001$), and hyphal length ($r = 0.73$, $P = 0.002$). Root growth of *E. camaldulensis* was more particularly negatively linked with the species richness of the herbaceous layer and with the soil catabolic evenness (Fig. 5).

Discussion

This study clearly shows that *E. camaldulensis* can significantly alter soil bacterial community when it has to compete for an AM fungus. Both the bacterial community structure (assessed by DGGE profiles) and function (assessed by SIR responses and soil catabolic evenness) were significantly affected. The changes in the bacterial structure and function were accompanied by changes in the composition of the herbaceous plant species layer. Since *Eucalyptus* plants in

Table 5. Catabolic evenness and average SIR responses ($\mu g\, CO_2\, g^{-1}\, soil\, h^{-1}$) with each substrate group (carboxylic acids, amino acids, amides and carbohydrates) in the four soil treatments

	Treatments			
	WEC*	Control	FA†	*Glomus intraradices*
Catabolic evenness	16.9 (0.62)‡ b§	17.8 (0.87) b	9.6 (0.51) a	20.7 (1.08) c
Carboxylic acids	236 (5.3) b	209 (10.4) a	293 (6.2) c	201 (11.6) a
Amino acids	131 (7.1) bc	107 (5.1) a	112 (7.5) ab	136 (9.1) c
Amides	191 (22.9) b	118 (9.7) a	120 (15.8) a	94.7 (14.4) a
Carbohydrates	239 (50.4) b	126 (9.1) a	117 (13.2) a	123 (10.9) a

*Without *Eucalyptus camaldulensis* seedlings.

†Preplanting fertilizer application.

‡SEM.

§Data in the line followed by the same letter are not significantly different according to Newman–Keuls test ($P < 0.05$).

association with *G. intraradices* did not heavily influence the soil biology and the structure of the herbaceous layer, the results of the present study highlighted the role of AM symbiosis and more particularly, the mycorrhizal soil infectivity, in the processes involved in soil bio-functioning and plant coexistence.

The effects of *Eucalyptus* spp. on soil quality vary greatly according to the type of site on which the plantations have been established. It has been shown that an *E. camaldulensis* monoculture plantation in a previously natural *Shorea robusta* forest site resulted in an increase in the soil pH but did not change soil fertility (Jha & Pande, 1984). In contrast, it has been reported that soil-chemical properties (organic carbon, total N, P and K) declined following reforestation with *Eucalyptus*, and further declined with plantation age (Bargali *et al*., 1993). Moreover, previous research has shown that exotic plant species can cause an increased pH in soils as well as higher nitrification rates and more available nitrate (Callaway, 1995; Kourtev *et al*., 1998, 1999, 2003; Ehrenfeld *et al*., 2001). In the present study, no significant changes in soil chemical characteristics have been recorded which could be explained by the relatively short duration of *E. camaldulensis* culture.

Our results showed that the development of herbaceous plant species was inhibited in the presence of *E. camaldulensis* plants (more particularly with non-AM-inoculated plants) and that this negative effect was linked with the *Eucalyptus* root growth. In field conditions, *Eucalyptus* drastically alters the annual vegetation by establishing gradients of toxicity in an otherwise relatively uniform environment (del Moral & Muller, 1970). These allelopathic interferences usually occur in the ecotone where *Eucalyptus* litter accumulates. In our experiment, the litter does not accumulate in the containers and the lack of herbs cannot result from the release of toxins from eucalyptus litter. It has been also reported that many compounds released from plant roots had deleterious effects on other plants (Inderjit & Mallik, 1997; Callaway & Aschehoug, 2000; Callaway, 2002). It suggests that these substances could act as allelochemicals as has been previously demonstrated with *Centaurea diffusa* and *C. maculosa*, which induce through their root exudates a strong negative effect on Bunchgrass species in North America (Callaway & Aschehoug, 2000).

In addition, the noninoculated *E. camaldulensis* plants have induced severe disturbances in soil microbial communities. These changes were mainly dependent on the extent of root growth. It has been well established that the structure and functional diversity of microbial communities in the soil were mainly dependent on aboveground plant composition (Grayston *et al*., 2001). More recently, it has been demonstrated that an invasive plant (*Alliaria petiolata*, garlic mustard) can disrupt native microbial communities by eliminating the activity of native AM fungi from the soil (Stinson *et al*., 2006). These authors showed that garlic mustard inhibited AM formation in native tree species through phytochemical inhibition, more particularly by reducing germination rates of native AM spores (Stinson *et al*., 2006). In the study, a similar process has been found as *Eucalyptus* drastically reduced mycorrhizal potentials and more particularly mycelial networks. These negative impacts could result from phytochemical inhibitions of AM fungal communities but also from *Eucalyptus* allelopathic effects on herbaceous plant species that, consequently, could not be involved in the development of native AM fungi. The experiment cannot separate a direct effect of root exudates on AM fungal communities and an indirect effect on AM fungi through a decrease of the herbaceous layer. However, root systems of *E. camaldulensis* were predominant in each container compared with the root extent of herbaceous plant species (more particularly in the preplanting fertilizer application treatment) and it could be assumed that the role of annual herbs could be negligible in AM formation and other microbial components.

Disturbances of AM soil infectivity were accompanied with a strong decrease of the soil catabolic evenness. It has been hypothesized that decreases in the microbial catabolic

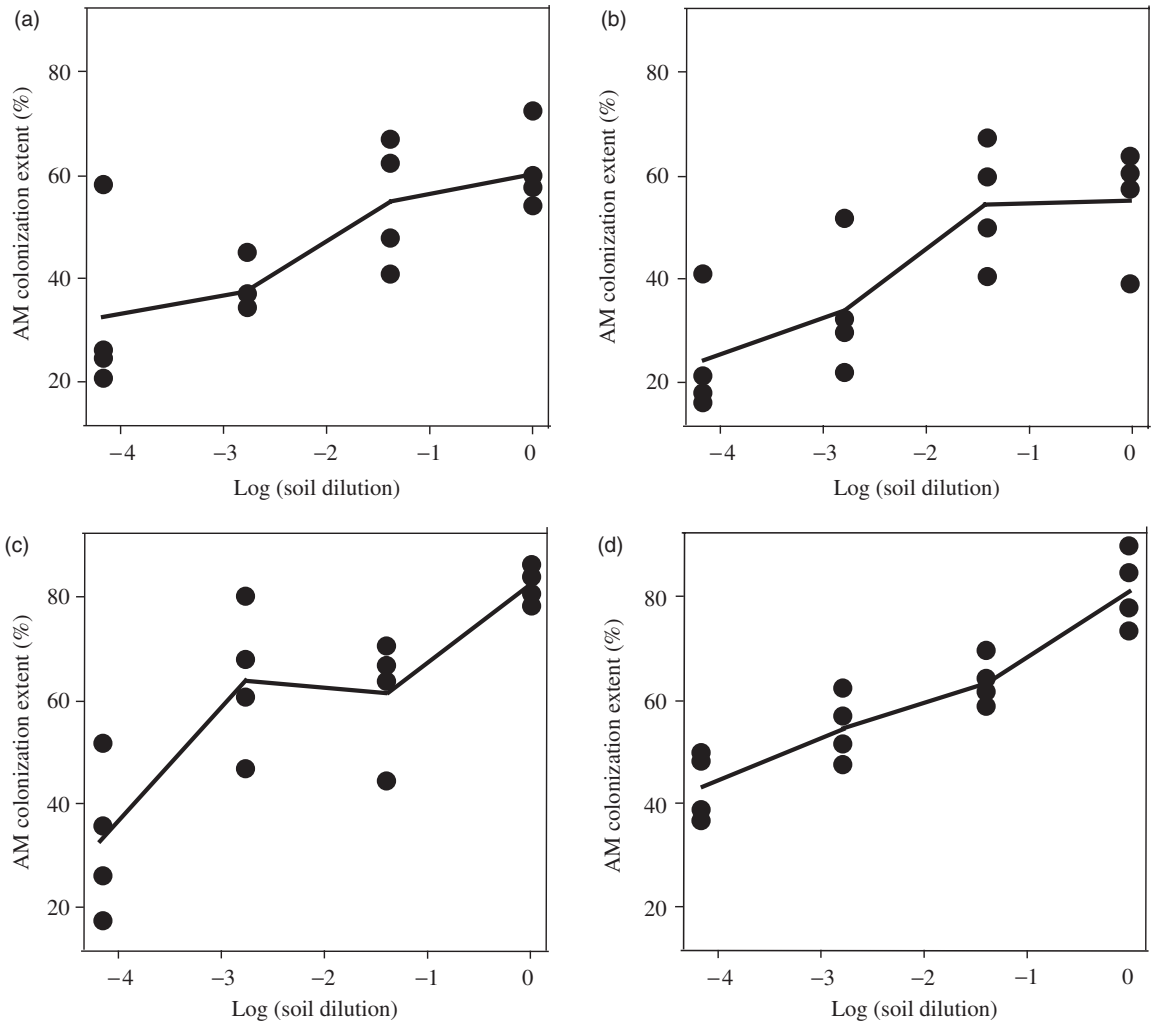

Fig. 4. Regression model between the extent of AM colonization and soil dilutions in each soil treatment. (a) Control; (b) preplanting fertilizer application; (c) AM inoculation with *Glomus intraradices*; (d) without *Eucalyptus camaldulensis* seedlings.

diversity will cause declines in the resistance of soils to stress or disturbances (Giller *et al.*, 1997). In the present study, the catabolic evenness of the soil was 16.9, which was in accordance with previous studies where catabolic evenness for soils under cropping ranged from 16.4 to 19.6 (Degens *et al.*, 2000). After 12 months' culture, fertilized *E. camaldulensis* plants had significantly decreased soil catabolic evenness to 9.6, which is rather low compared to the literature (Degens *et al.*, 2000). In addition, changes in microbial catabolic evenness were related to changes in the structure of bacterial communities. The functional diversity of microbial communities includes a vast range of activities as nutrient transformation, decomposition, plant growth promotion and modification of soil physical processes (Wardle *et al.*, 1999). The measurement of catabolic response profiles assesses the catabolic diversity of microbial communities involved in decomposition activities (Degens *et al.*, 2000). The data suggest that *E. camaldulensis* depleted this catabolic diversity by reducing or eliminating some components of soil microbial communities involved in the decomposition of organic C fraction. A higher average SIR response with carboxylic acids was also recorded from the soils collected under fertilized *Eucalyptus*. Organic acids increase the solubility and rate of inorganic P uptake (Grayston *et al.*, 1996). It is well known that this way of phosphate acquisition is important for plants adapted to acid mineral soils with very low inorganic phosphate availability, such as for *Eucalyptus* spp. (Mulette *et al.*, 1974). Hence, large amounts of carboxylic acids could exert a selective influence on soil microbial communities through a multiplication of carboxylic acid catabolizing microorganisms while inducing a higher SIR response.

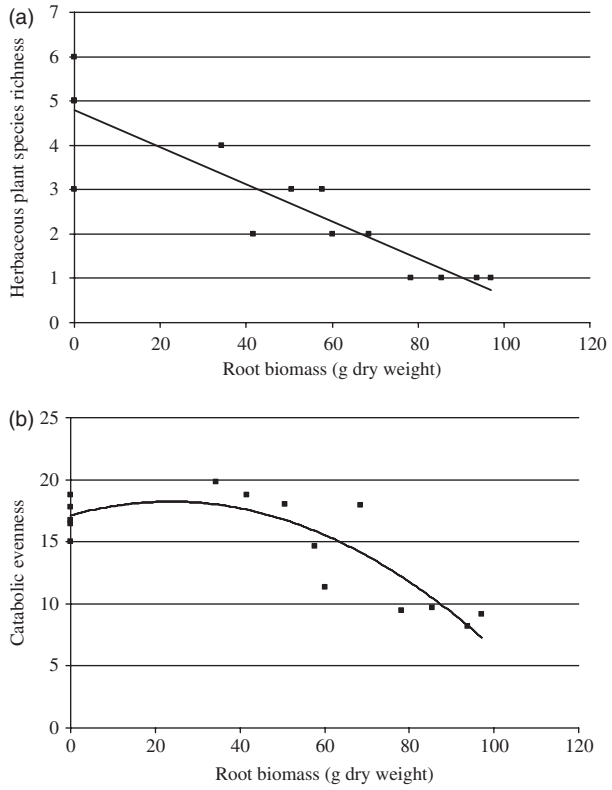

Fig. 5. Correlations between root biomass of noninoculated *Eucalyptus camaldulensis* plants (control and preplanting fertilizer application treatments) and (a) herbaceous plant species richness ($y = 4.8–0.42x$, $r = 0.91$, $P < 0.0001$) and (b) soil catabolic evenness ($y = -0.002x^2 + 0.096x + 117.1$, $r = 0.86$, $P = 0.0003$).

The inoculation of *G. intraradices* is highly beneficial to the growth of *E. camaldulensis* in the disinfected sandy soil, and this stimulating effect is kept during 12 months' culture in containers filled with the same soil but nondisinfected. This result is in accordance with other studies, from which it has been established that this fungal isolate was very efficient on the growth of other plant species (Villenave *et al.*, 2003; Duponnois & Plenchette, 2003; Duponnois *et al.*, 2005). Moreover, the fungal inoculation tends to return the soil to its initial conditions with a similar bacterial community structure and higher catabolic evenness and soil mycorrhizal potential. AM symbiosis generally increases root exudation (Grayston *et al.*, 1996), influences carbohydrate metabolism of the host plant (Shachar-Hill *et al.*, 1995) and influences rhizosphere microbial communities (Johansson *et al.*, 2004). AM hyphae exude chemical compounds that have a selective effect on the microbial communities in the rhizosphere and in the soil (Andrade *et al.*, 1998). Together, these microbial compartments are commonly named 'mycorrhizosphere' (Linderman, 1988). From the present study, it is clearly demonstrated that this AM fungal inoculation effect on the composition and function of soil microbial communities was not root growth dependent but was mainly due to the presence of the AM fungus and more particularly to the extramatrical mycelium. Van der Heijden *et al.* (1998) argued that an increase in hyphal lengths led to a higher plant diversity, ecosystem variability and productivity. These results underline the importance of the extramatrical mycelium in soil microbial interactions and in the evolution of ecosystems. It has been suggested that catabolic evenness was an integrative indicator of the susceptibility of microbial communities to changes in soil conditions (Degens *et al.*, 2001). Hence, soils with high catabolic evenness are more resistant to stress and disturbance. The results highlight the importance of AM symbiosis in the functioning of soils and its importance in sustainable agriculture (Jeffries *et al.*, 2003).

In addition, AM inoculation has increased the development of herbaceous plant species under inoculated *E. camaldulensis* plants. This positive effect could result from the well-developed mycelial network by equalizing the distribution of soil resources among competitively dominant and subdominant species (Wirsel, 2004). But it is also known that microorganisms can act as allelochemical mediators, inactivating or metabolizing toxic compounds and it has been suggested that AM fungi, associated with their mycorrhizosphere microbial communities, could protect seedlings from allelopathy (Pellissier & Souto, 1999; Blum *et al.*, 2000; Renne *et al.*, 2004).

In conclusion, this study shows that exotic plant species can drastically affect soil microbial community and alter both the community structure and function. These changes are significantly correlated with the antagonistic effects of *E. camaldulensis* against herbaceous plant species. The negative impact of this exotic tree species is significantly modified when it is inoculated with an efficient AM fungus. Although these biological interactions are clearly demonstrated from the present study, field-based experimental research must be undertaken to determine the impact of AM potential on the interactions between plant species, especially between exotic and native plant species.

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References

Andrade G, Mihara KL, Linderman RG & Bethlenfalvay GJ (1998) Soil aggregation status and rhizobacteria in the mycorrhizosphere. *Plant Soil* **202**: 89–96.

Assigbetse K, Gueye M, Thioulouse J & Duponnois R (2005) Soil bacterial diversity responses to root colonization by an

ectomycorrhizal fungus are not root-growth dependent. *Microbial Ecol* **50**: 350–359.
Bargali SS, Singh RP & Joshi M (1993) Changes in soil characteristics in eucalypt plantations replacing natural broad-leaved forests. *J Veg Sci* **4**: 25–28.
Blum U, Statman KL, Flint LJ & Shaefer SR (2000) Induction and/or selection of phenolic acid-utilizing bulk-soil and rhizospheric bacteria and their influence on phenolic acid phytotoxicity. *J Chem Ecol* **26**: 2059–2078.
Brundrett MC, Piche Y & Peterson RL (1985) A developmental study of the early stages in vesicular-arbuscular mycorrhizal formation. *Can J Bot* **63**: 184–194.
Callaway RM (1995) Positive interactions among plants. *Bot Rev* **61**: 306–349.
Callaway RM (2002) The detection of neighbors by plants. *Trends Ecol Evol* **17**: 104–105.
Callaway RM & Aschehoug ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic evasion. *Science* **290**: 521–523.
Callaway RM & Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Front Ecol Environ* **2**: 436–443.
Chippendale GM (1988) Flora of Australia, Volume 19, Myrtaceae, *Eucalyptus*, *Angophora*. Australian Government Publishing Services, Canberra.
Costermans LF (1989) Native trees and shrubs of south-eastern Australia, Weldon, Sydney.
Couto L & Betters DR (1995) *Short Rotation Eucalypt Plantations in Brazil: Social and Environmental Issues*. Oak Ridge National Laboratory, Oak Ridge, TN.
Culhane AC, Perriere G, Considine EC, Cotter TG & Higgins DG (2002) Between-group analysis of microarray data. *Bioinformatics* **18**: 1600–1608.
Degens BP & Harris JA (1997) Development of a physiological approach to measuring the metabolic diversity of soil microbial communities. *Soil Biol Biochem* **29**: 1309–1320.
Degens BP & Vojvodic-Vukovic M (1999) A sampling strategy to assess the effects of land use on microbial functional diversity in soils. *Aust J Soil Res* **37**: 593–601.
Degens BP, Schipper LA, Sparling GP & Vojvodic-Vukovic M (2000) Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil Biol Biochem* **32**: 189–196.
Degens BP, Shipper LA, Sparling GP & Duncan LC (2001) Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? *Soil Biol Biochem* **33**: 1143–1153.
del Moral R & Muller CH (1970) The allelopathic effects of *Eucalyptus camaldulensis*. *Am Midland Nat* **83**: 254–282.
Dolédec S & Chessel D (1989) Rythmes saisonniers et composantes stationnelles en milieu aquatique II- Prise en compte et élimination d'effets dans un tableau faunistique. *Acta Oecol* **10**: 207–232.
Duponnois R & Plenchette C (2003) A mycorrhiza helper bacterium (MHB) enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species. *Mycorrhiza* **13**: 85–91.
Duponnois R, Colombet A, Hien V & Thioulouse J (2005) The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. *Soil Biol Biochem* **37**: 1460–1468.
Ehrenfeld J, Kourtev P & Huang W (2001) Changes in soil functions following invasions of exotic understory plants in deciduous forests. *Ecol Appl* **11**: 1287–1300.
Evans J (1982) *Plantation Forestry in the Tropics*. Clarendon Press, Oxford.
Giller KE, Beare MH, Lavelle P, Izac A-MN & Swift MJ (1997) Agricultural intensification, soil biodiversity and agroecosystem function. *Appl Soil Ecol* **6**: 3–16.
Grayston SJ, Vaughan D & Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Appl Soil Ecol* **5**: 29–56.
Grayston SJ, Griffith GS, Mawdsley JL, Campbell CD & Bardgett RD (2001) Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biol Biochem* **33**: 533–551.
Heinemeyer O, Insam H, Kaiser EA & Walenzik G (1989) Soil microbial biomass and respiration measurements: an automated technique based on infrared gas analysis. *Plant Soil* **116**: 77–81.
Hobbs RJ & Huenneke LF (1992) Disturbance, diversity and invasion: Implications for conservations. *Conserv Biol* **6**: 324–337.
Huberty CJ (1994) *Applied Discriminant Analysis*. John Wiley & Sons, New York.
Inderjit K & Mallik AU (1997) Effect of phenolic compounds on selected soil properties. *Forest Ecol Manage* **92**: 11–18.
Jakobsen I & Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytol* **115**: 77–83.
Jeffries P, Gianinazzi S, Perotto S, Turnau K & Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil Soils* **37**: 1–16.
Jha MN & Pande P (1984) Impact on growing *Eucalyptus* and sal monocultures on soil in natural sal area of Doon Valley. *Indian Forester* **110**: 16–22.
Johansson JF, Paul LR & Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol Ecol* **48**: 1–13.
Klironomos JN, McCune J, Hart M & Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecol Lett* **3**: 137–141.
Kourtev P, Ehrenfeld J & Huang W (1998) Effects of exotic plant species on soil properties in hardwood forests of New Jersey. *Water Air Soil Pollut* **105**: 493–501.

Kourtev P, Huang W & Ehrenfeld J (1999) Differences in earthworm densities and nitrogen dynamics in soils under exotic and native plant species. *Biol Inv* **1**: 237–245.

Kourtev P, Ehrenfeld J & Häggblom M (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biol Biochem* **35**: 895–905.

Krebs CJ (1989) *Ecology Methodology*. Harper Collins Publishers, New York.

Levine JM & D'Antonio CM (1999) Elton revisited: a review of evidence linking diversity and invasibility. *Oikos* **87**: 15–26.

Linderman RG (1988) Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* **78**: 366–371.

Magurran AE (1988) *Ecological Diversity and its Measurement*. Croom Helm, London.

Marler MJ, Zabinski CA & Callaway RM (1999) Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* **80**: 1180–1186.

Mooney HA & Hobbs RJ (2000) *Invasive Plants in a Changing World*. Island Press, Washington, DC.

Mulette KL, Hannon NJ & Elliot AGL (1974) Insoluble phosphorus usage by *Eucalyptus*. *Plant Soil* **41**: 199–205.

Muyzer G, De Waal EC & Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain-reaction-amplified genes coding for 16S rRNA. *Appl Environ Microb* **59**: 695–700.

N.A.S. (1980) *Firewood Crops. Shrub and Tree Species for Energy Production*. National Academy of Sciences, Washington, DC.

O'Connor PJ, Smith SE & Smith FA (2002) Arbuscular mycorrhizas influence plant diversity and community structure in a semiarid herbland. *New Phytol* **154**: 209–218.

Ovreas L, Forney L, Daae FL & Torsvik V (1997) Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl Environ Microbiol* **63**: 3367–3373.

Parrotta JA (1993) Secondary forest regeneration on degraded tropical lands. The role of plantations as "foster ecosystems". *Restoration of Tropical Forest Ecosystems* (Leith H & Lohmann M, eds), pp. 63–73. Kluwer Academic Publishers.

Pellissier F & Souto X (1999) Allelopathy in Northern temperate and boreal semi-natural woodland. *Crit Rev Plant Sci* **18**: 637–652.

Phillips JM & Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* **55**: 158–161.

Pinheiro JC & Bates DM (2000) *Mixed-Effects Models in S and S-PLUS*. Springer, New York.

R Development Core Team (2006) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rejmanek M (2000) Invasive plants: approaches and predictions. *Aust Ecol* **25**: 497–506.

Renne IJ, Rios BG, Fehmi JS & Tracy BF (2004) Low allelopathic potential of an invasive forage grass on native grassland plant: a cause for encouragements? *Basic Appl Ecol* **5**: 261–269.

Richardson DM, Allsopp N, D'Antonio CM, Milton SJ & Rejmanek M (2000) Plant invasion–the role of mutualisms. *Biol Rev Camb Philos Soc* **75**: 65–93.

Schreiner RP & Bethlenfalvay GJ (1995) Mycorrhizal interactions in sustainable agriculture. *Crit Rev Biotech* **15**: 271–285.

Schreiner RP, Mihara KL, Mc Daniel H & Benthlenfalvay GJ (2003) Mycorrhizal fungi influence plant and soil functions and interactions. *Plant Soil* **188**: 199–209.

Shachar-Hill Y, Pfeffer PE, Douds D, Osman SF, Doner LW & Ratcliffe RG (1995) Partitioning of intermediary carbon metabolism in vesicular-arbuscular mycorrhizal leeks. *Plant Physiol* **108**: 7–15.

Shine C, Williams N & Gundling L (2000) *A guide to designing legal and institutional frameworks on alien invasive species. Vol. 40*. IUCN, Switzerland.

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D & Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLOS Biol* **4**: 727–731.

Thébaud C & Simberloff D (2001) Are plants really larger in their introduced ranges? *Am Nat* **157**: 231–236.

Thioulouse J, Chessel D, Dolédec S & Olivier JM (1997) ADE-4: a multivariate analysis and graphical display software. *Stat Comput* **7**: 75–83.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A & Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 72–75.

van der Heijden MGA, Wiemken A & Sanders IR (2003) Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occuring plant. *New Phytol* **157**: 569–578.

Venables WN & Ripley BD (2002) *Modern Applied Statistics*. Springer, New York.

Villenave C, Leye K, Chotte JL & Duponnois R (2003) Nematofauna associated with the mycorrhizosphere and hyphosphere of *Glomus intraradices* and exotic or native leguminous plant species mycorrhizal formation in West Africa. *Biol Fert Soils* **38**: 161–169.

Wardle DA, Giller KE & Barker GM (1999) The regulation and functional significance of soil biodiversity in agro-ecosystems. *Agrobiodiversity: Characterisation, Utilisation and Management* (Wood D & Lenné JM, eds), pp. 87–121. CABI, London.

West HM (1996) Influence of arbuscular mycorrhizal infection on competition between *Holcus lanatus* and *Dactylis glomerata*. *J Ecol* **84**: 429–438.

West AW & Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurements of microbial biomass in soils of different water contents. *J Microbiol Meth* **5**: 177–189.

Wirsel SGR (2004) Homogenous stands of wetland grass harbour dicerse consortia of arbuscular mycorrhizal fungi. *FEMS Microb Ecol* **48**: 129–138.